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STAIN TECHNOLOGY
Vol. 51, No. 1

Printed in U.S.A.

SPECIMEN HOLDERS FOR SIMULTANEOUS CRITICAL POINT DRYING OF MULTIPLE BIOLOGICAL SPECIMENS

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ABSTRACT **Specimen holders were developed for simultaneous critical point drying of multiple specimens of monolayer cell cultures grown on Leighton tube glass cover slips or for specimens of cells collected on silver or cellulose membrane filters. The use of these multiple specimen holders makes it possible to process several specimens in parallel and thus significantly reduce the time required for handling large numbers of samples and also eliminate the possible variations that occur in processing individual samples one at a time in series.**

This laboratory is engaged in testing of polymeric materials for biodegradation and biocompatibility and in the development of *in vitro* methods for prescreening of these materials for any bio-interaction. In an effort to correlate the possible effects of sublethal doses of toxic polymeric degradation products on cell morphology and ultrastructure, we have used the critical point drying method of Anderson (1951) or a modification of it (De Bault 1973, Lewis and Nemanic 1973). This is the mildest method known for biological specimen drying and is considered to induce only minimal shrinkage and surface alterations (Boyde 1971).

In our *in vitro* screening system, test substances are placed into monolayer cell cultures grown on Leighton tube glass cover slips. At the end of the test period, most of the cells are still attached to the cover slips; however, depending upon the extent of cytotoxicity of the test material, some cells are detached and float freely in the medium. These free floating cells are collected on Flotronics (Selas Corporation of America, Spring House, PA) or Millipore (Millipore Corporation, Bedford, MA) filters. Because of the lack of availability of multiple specimen holders for critical point drying of cells grown on Leighton tube cover slips, and since the holders for membrane filter preparations from the different manufacturers were not the proper size to fit the Parr drying bomb, two different types of holders were constructed in our laboratory. The two holders discussed in this article were constructed to hold several of either the glass cover slips or the filters during the critical point drying process.

The holder in Figure 1 can be fitted into the Parr 4770 critical point drying apparatus. It holds 22 glass cover slips 9 mm × 50 mm and was made from two pieces of ⅜ × 2 × 2 inch flat stock machined to obtain the desired dimensions. The two halves were welded together and then welded to a 2⅛-inch diameter circular bottom. A pin was placed through the bottom of the holder to keep the cover slips from falling through. The two solid side pieces were machined from 2⅛-inch diameter stock and were incorporated into the design to reduce dead space and thus the amount of liquid used. There is a ⅜-inch diameter hole in one side piece to accommodate the exhaust

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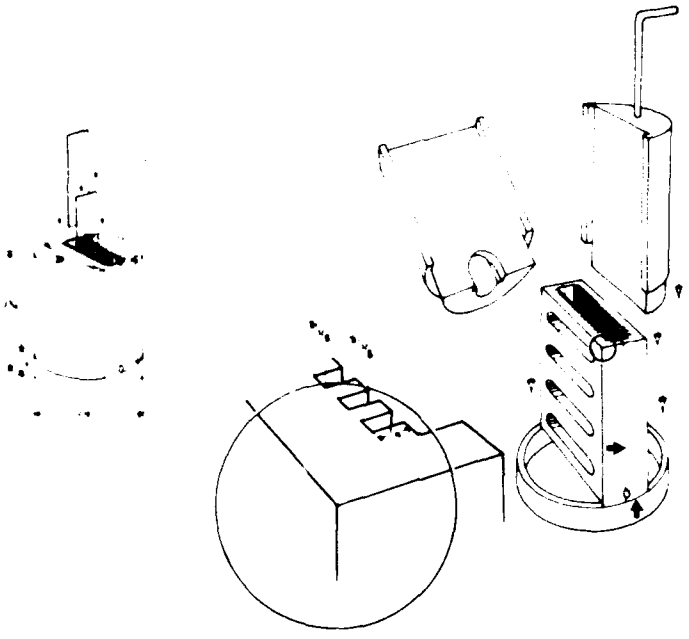

FIG. 1. Aluminum holder for critical point drying of cells on Leighton tube glass cover slips. Large arrows show welded joints, and the slots in the side of the slide holder are to assure adequate circulation of fluids in sample preparation (see text).

tube of the Parr bomb and to allow the use of a pipette for fluid changes. A handle was placed on the other side piece to facilitate removal; however, this could be placed on the holder itself.

Figure 2 shows the holder, which also fits the Parr apparatus, used for the preparation of specimens on membrane filters. All sections were machined from 2 1/8-inch diameter stock, and the sample supports were cut from 100 mesh stainless steel screen. The design permits each section to fit inside the rim of the section below it, and the greatest number of sections that can be stacked is determined by the length of the supporting rod. The 3/8-inch diameter hole is needed to accommodate the exhaust tube as shown in the inset of Fig. 2. This holder has proven to be very versatile and samples other than the membrane filters have been processed successfully. The size of the specimens accommodated by this holder is limited by the indicated dimensions.

Our routine for critical point drying with these holders is as follows: The biological material on the glass slides or Flotronics filters is placed in the special holder, fixed in 2% glutaraldehyde for 2 hr, washed with 0.15 M phosphate buffer, fixed in 2% osmium tetroxide at 0 C for 30 min, washed twice with 0.15 M phosphate buffer, and dehydrated with ethanol (50%, 75%, 95%, 100%, 100%) in 10-min steps. The ethanol is then replaced in 10-min steps with increasing concentrations of isoamyl acetate (50%, 75%, 95%, 100%, 100%).

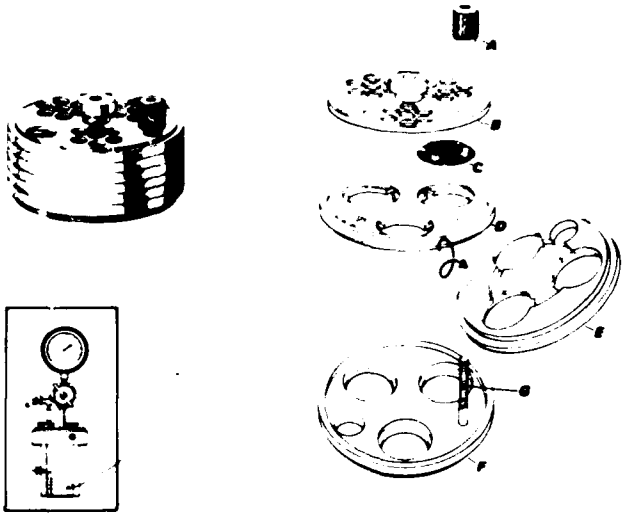

FIG. 2. Aluminum holder for critical point drying of cells on membrane filters. This holder is comprised of several $\frac{3}{8}$ inch thick sections, (*D*, *E*, and *F*) the number of which depends on the length of the threaded post (*G*). In each section there is one $\frac{1}{8}$ inch hole for the post (*G*) one $\frac{1}{8}$ inch hole to accommodate the exhaust tube of the Parr system, and three $\frac{3}{4}$ inch holes, each with a $\frac{1}{16}$ inch lip to hold a $\frac{23}{32}$ inch diameter 100 mesh stainless steel screen (*C*). The bottom of each individual section (*E*) has a $\frac{1}{8}$ inch groove connecting the $\frac{3}{4}$ inch holes. The top (*B*) is 2 inch in diameter, has a centrally placed handle and has the same holes as the individual sections, except the three $\frac{3}{4}$ inch holes are replaced by numerous $\frac{1}{8}$ inch holes. Nut (*A*) is screwed onto post (*G*) to hold all sections in place as shown in upper left drawing. Inset shows a cutaway view of the complete Parr system with a holder in place. An arrow indicates the exhaust tube.

The samples in the holder are then transferred to a Parr critical point drying apparatus and critical point dried with liquid CO_2 as the transitional fluid, according to the method described by Anderson. Approximately 9 × 9 mm squares of either the Flotronics filters or the glass cover slips are mounted on aluminum stubs with All Purpose Cement No. 527 (Bond Adhesives Co., Jersey City, N.J.) and conductive paint, and evaporation coated with carbon and gold in a VE 10 vacuum evaporator (Varian Vacuum Division, Palo Alto, CA) at 2×10^{-5} Torr (Echlin 1974). The specimens are examined in a Model 1000 scanning electron microscope (Advanced Metals Research Corp., Burlington, MA) with an accelerating voltage of 20 keV and a resolution of 100-200Å. Samples collected on Millipore filters are soluble in this intermediate fluid, and are therefore critical point dried according to De Bault's modification of Anderson's method.

We have obtained excellent results using these two holders for the critical point drying process. The use of these holders has decreased the time necessary for processing multiple samples.

Another advantage of processing samples in these holders is that any differences between individual sample preparations due to minor variations in procedure are eliminated.

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SECURITY CLASSIFICATION OF THIS PAGE *(When Data Entered)*

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) SPECIMEN HOLDERS FOR SIMULTANEOUS CRITICAL POINT DRYING OF MULTIPLE BIOLOGICAL SPECIMENS.		5. TYPE OF REPORT & PERIOD COVERED
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Rice, Robert M.; Hegyeli, Andrew F. and Breeden, Luther G., Jr.		8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Medical Bioengineering Research & Development Laboratory, Bldg. 568, Ft. Detrick, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS In vitro Testing
11. CONTROLLING OFFICE NAME AND ADDRESS		12. REPORT DATE
		13. NUMBER OF PAGES 4
14. MONITORING AGENCY NAME & ADDRESS *(if different from Controlling Office)*		15. SECURITY CLASS. *(of this report)* Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT *(of this Report)*

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT *(of the abstract entered in Block 20, if different from Report)*

18. SUPPLEMENTARY NOTES

Published in STAIN TECHNOLOGY 51: No. 1, pp 51-53 (1976)

19. KEY WORDS *(Continue on reverse side if necessary and identify by block number)*

Scanning electron microscopy
Critical point drying
Tissue culture

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

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